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(57) Abstract

This invention provides methods for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor or by detecting the presence of an Id protein in the tumor and a method for *in situ* identification of tumors which may be susceptible to treatment by chemotherapy or radiation. This invention also provides antisense oligonucleotides, a pharmaceutical composition comprising an amount of the oligonucleotide, methods of inducing terminal differentiation in a tumor cell, and methods of treating a tumor in a subject.

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Id AS A DIAGNOSTIC MARKER IN TUMOR CELLS

The invention disclosed herein was made in the course of work under NCI Core Grant No. 08748 from the National Cancer Institute and Grant No. IBN-9228977 from the National Science foundation. The U.S. Government has certain rights in this invention.

Background of the Invention

A sequence motif common to a number of proteins involved in cell type determination or transcriptional regulation has recently been described. The motif was initially identified as a region of homology among *c-myc*, the muscle determination gene MyoD (Davis et al., 1987), and genes of the Drosophila *acheaete-scute* complex involved in neural determination (Villares and Cabrera, 1987 and references therein). This region has now been found in the muscle determination genes Myf-5 (Braun et al. 1989) and mycgenin (Edmonson and Olson, 1989, Wright et al., 1989) as well as other genes involved in cell type determination in Drosophila such as daughterless (Cronmiller et al., 1988, Caudy et al., 1988) hairy (Rushlow et al. 1989), twist (Thisse et al. 1988), and Enhancer of split (Klambt et al., 1989). Other genes in the family include two human κ immunoglobulin enhancer binding proteins, E12 and E47 (Murre et al., 1989a), and lyl-1, a gene found at the breakpoint of a chromosome translocation present in two human T cell leukemia cell lines (Millentin and Cleary, 1989).

This homology region has been divided into two conceptual subdomains: the helix-loop-helix (HLH) domain (Murre et al., 1989a) and an adjacent basic region (Tapscott et al. 1988). Reference is made to the combined motif as the B-HLH region. The HLH domain consists of two segments capable of forming amphipathic α helices (12-15 residues

each) connected by a nonconserved "loop" region of varying length (Murre et al., 1989a). The basic regions, which is just amino-terminal to the HLH domain, is 10-20 amino acids in length and consists of two to three clusters of basic amino acid residues.

Various proteins that have a B-HLH region can form homodimeric and heterodimeric complexes in vitro (Murre et al., 1989a, 1989b; Davis et al., 1990). The HLH domain of MyoD has been shown recently to be necessary for the formation of MyoD-E12 heterodimers (Davis et al., 1990). These heterodimers bind to a sequence present in the muscle creatine kinase (MCK) enhancer and the κ light chain enhancer with much higher affinity than either species alone (Davis et al. 1990; Murre et al., 1989b). The HLH domain is therefore at least indirectly responsible for high affinity DNA binding. In the case of MyoD, the basic region is not required for heterodimer formation but is required for specific DNA binding and myogenic activity in vivo (Davis et al., 1990). In fact, expression of a 68 amino acid peptide of MyoD that includes just the basic region and the HLH domain has been shown to be sufficient for both DNA binding in vitro (Lassar et al., 1989) and for myogenesis in a stably trasfected mouse embryo fibroblast line (Tapscott et al., 1988).

Benezra et al. reported the isolation of a different type of HLH gene using a probe from the conserved region of amphipathic helix 2 (Benezra et al. 1990). The predicted protein product encoded by this clone is distinct in that the basic region adjacent to the HLH domain is absent. It was demonstrated that the protein can specifically associate with three other members of the HLH family (Myo-D, E12 and E47) and attenuate their ability to bind specific DNA sequences. In addition forced expression of the gene in transfection experiments was shown to inhibit

Myo-D dependent activation of the MCK enhancer. The clone was labeled Id for "inhibitor of DNA binding."

Thus far, two other human Id clones in addition to Id1 have been identified, namely Id2 and Id3. (Biggs, J. et al., 1992; Ellmeier, W. et al., 1992). All three Id clones have a high degree of amino acid homology within the HLH domain but diverge outside this domain. Probes specific for each Id gene can therefore be designed by targeting regions outside of the HLH domain.

As discussed more fully below, Id is expressed in tumor cells. The expression of Id in tumors can be reduced and, thereby, the tumors can be induced to undergo normal differentiation and stop their proliferation.

Summary of the Invention

This invention provides a method for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor which method comprises:

a) contacting a tumor sample with a nucleic acid probe, wherein the probe is labeled with a detectable moiety and comprises at least 50 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of nucleic acid encoding an Id gene, under conditions permitting the hybridization of the probe to any of the nucleic acid present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any probe not hybridized in the tumor sample; and

c) detecting the presence of any probe hybridized to any of the nucleic acid present in the tumor sample thereby detecting the presence of nucleic acid encoding Id in the tumor sample.

This invention also provides a method for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of an Id protein in the tumor which method comprises:

a) contacting a tumor sample with an antibody, wherein the antibody is labeled with a detectable moiety and is capable of specifically binding to an Id protein, under conditions permitting the binding of the antibody to any of the protein present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any antibodies not bound in the tumor sample; and

c) detecting the presence of any antibodies bound to the protein in the tumor sample thereby detecting the presence of an Id protein in the tumor.

This invention also provides a method for in situ identification of tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor which method comprises contacting the tumor with a suitably labeled nucleic acid reagent capable of detecting an Id gene or gene product.

This invention further provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA. This invention also provides a replicable vector comprising the antisense oligonucleotide and a host cell containing the vector.

This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA which is capable of passing through a cell membrane and effective to inhibit the expression of Id and a suitable pharmaceutically acceptable carrier.

This invention also provides a method of inducing terminal differentiation in a tumor cell which comprises contacting the tumor cell with the antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA.

This invention also provides a method of treating a tumor in a subject which comprises administering the antisense

oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA.

This invention also provides a method of treating a tumor in a subject which comprises administering the pharmaceutical composition defined above to the subject.

Brief Description of the Figure

Figures 1A-C: Upregulation of Id2 in Rhabdomyosarcoma (RMS) tumor cell line. Total RNA from the cell lines indicated was isolated while the cells were growing in 15% FCS (g) or 48 hours after mitogen depletion (d). The same Northern filter was hybridized to human probes specific for Id1 (Figure 1A), Id2 (Figure 1C) and Id3 (Figure 1B) as indicated. RH1, RH30, RH18, RH28, and HS729 are all derived from primary RMS. PHM refers to early passage primary human myoblasts (gift of Dr. Stephen Tapscott).

Detailed Description

This invention provides a method for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor which method comprises:

a) contacting a tumor sample with a nucleic acid probe, wherein the probe is labeled with a detectable moiety and comprises at least 50 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of nucleic acid encoding an Id gene, under conditions permitting the hybridization of the probe to any of the nucleic acid present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any probe not hybridized in the tumor sample; and

c) detecting the presence of any probe hybridized to any of the nucleic acid present in the tumor sample thereby detecting the presence of nucleic acid encoding Id in the tumor sample.

In one embodiment of this invention the Id gene may be the Id1, Id2 or Id3 gene. In a preferred embodiment the Id gene is the Id2 gene. In the practice of the above-described method, the nucleic acid probe may comprise between 50 and 300 nucleotides. In a preferred embodiment, the nucleic acid probe comprises about 150 nucleotides.

The term "probe" as used herein refers to any nucleic acid molecule which can be labeled and which forms a double helix by binding with a molecule containing a nucleic acid sequence of interest through complementary base paring. Those skilled in the art also refer to such probes as "hybridization probes." For example, when

using a DNA probe to locate a DNA sequence of interest, a sample containing double stranded DNA can be reacted with the DNA probe to locate any DNA molecule in a sample which comprises the sequence of interest ("target DNA"). In such methods, the double stranded DNA in the sample is disassociated into its single strands and then reacted with a DNA probe. The probe binds to any target DNA in the sample by complementary base paring, i.e., adenine matches with thymidine and guanine with cytosine. The DNA probe, therefore, is a single strand of a DNA double helix which comprises nucleic acid molecules which are complementary to the sequence of interest.

Methods of making labeled nucleic acid probes, both DNA and RNA, are well known to those of ordinary skill in the art.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence sufficiently similar to its own so as to form double-helical segments through hydrogen bonding between complementary base pairs. As used herein, a "unique sequence" is a sequence specific to only the nucleic acid molecules encoding an Id protein.

In the practice of the above-described method the detectable moiety may be a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label or a label which may be detected through a secondary enzymatic or binding step. The secondary enzymatic or binding step may comprise the use of digoxigenin, alkaline phosphatase, horseradish peroxidase, ß-galactosidase, fluorescein or steptavidin/biotin.

This invention also provides a method for identifying tumors which may be susceptible to treatment by

chemotherapy or radiation by detecting the presence of an Id protein in the tumor which method comprises:

a) contacting a tumor sample with an antibody, wherein the antibody is labeled with a detectable moiety and is capable of specifically binding to an Id protein, under conditions permitting the binding of the antibody to any of the protein present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any antibodies not bound in the tumor sample; and

c) detecting the presence of any antibodies bound to the protein in the tumor sample thereby detecting the presence of an Id protein in the tumor.

In the practice of the above-described method, the Id protein may be the Id1, Id2 or Id3 protein. In a preferred embodiment, the Id protein is the Id2 protein.

The production of antibodies to the proteins of this invention is within the skill of one of ordinary skill in the relevant art. In the practice of the above-described method, the antibodies may be polyclonal or monclonal antibodies. In a preferred embodiment the antibodies are monoclonal antibodies.

Polyclonal antibodies may be produced by injecting a host animal such as rabbit, rat, goat, mouse or other animal with the protein of this invention. The sera are extracted from the host animal and are screened to obtain polyclonal antibodies which are specific to the immunogen. Methods of screening for polyclonal antibodies are well known to those of ordinary skill in the art such as those disclosed in Harlow & Lane, Antibodies: A Laboratory Manual, (Cold Spring Harbor

Laboratories, Cold Spring Harbor, NY: 1988) the contents of which are hereby incorporated by reference.

Monoclonal antibodies may be produced by immunizing for example, mice with an immunogen. The mice are inoculated intraperitoneally with an immunogenic amount of the protein of the invention and then boosted with similar amounts of the protein. Spleens are collected from the immunized mice a few days after the final boost and a cell suspension is prepared from the spleens for use in the fusion.

Hybridomas may be prepared from the splenocytes and a murine tumor partner using the general somatic cell hybridization technique of Kohler, B. and Milstein, C., Nature (1975) 256: 495-497. Available murine myeloma lines, such as those from the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, MD 20852 USA, may be used in the hybridization. Basically, the technique involves fusing the tumor cells and splenocytes using a fusogen such as polyethylene glycol. After the fusion the cells are separated from the fusion medium and grown in a selective growth medium, such as HAT medium, to eliminate unhybridized parent cells. The hybridomas may be expanded, if desired, and supernatants may be assayed by conventional immunoassay procedures, for example radioimmunoassay, using the immunizing agent as antigen. Positive clones may be characterized further to determine whether they meet the criteria of the invention antibodies.

Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, as the case may be, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis,

chromatography, and ultrafiltration, if desired.

In the practice of the above-described method the detectable moiety may be a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label or a label which may be detected through a secondary enzymatic or binding step. The secondary enzymatic or binding step may comprise the use of digoxigenin, alkaline phosphatase, horseradish peroxidase, ß-galactosidase, fluorescein or steptavidin/biotin.

This invention also provides a method for in situ identification of tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding the Id gene in the tumor which method comprises contacting the tumor with a suitably labeled reagent capable of detecting an Id gene or gene product.

In the practice of the above-described method, the Id gene may be the Id1, Id2 or Id3 gene. The reagent may be an oligonucleotide having a sequence

In the practice of the above-described method the suitably labeled nucleic acid reagent may be labeled with a detectable moiety chosen from the group consisting of a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label and a label which may be detected through a secondary enzymatic or binding step.

This invention further provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA.

In a preferred embodiment of the antisense oligonucleotide, the oligonucleotide has a sequence capable of specifically hybridizing to an mRNA encoding for the Id2 gene.

The subject invention also provides a replicable vector comprising the antisense oligonucleotide. In the practice of this invention the vector may be contained within a host cell. In one embodiment the host cell is a eukaryotic cell. In another embodiment the host cell is a bacterial cell. In still another embodiment the host cell is a mammalian cell.

This invention also provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA which is capable of passing through a cell membrane and effective to inhibit the expression of Id and a suitable pharmaceutically acceptable carrier.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, water, emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stensic acid, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may also include flavor and color additives or other ingredients.

In addition to the standard characteristics of the pharmaceutically acceptable carriers, the "suitable"

carriers of the subject are further characterized as being able to penetrate the cell membrane. Therefore in one embodiment of the pharmaceutical composition the pharmaceutically acceptable carrier binds to a receptor on a cell capable of being taken up by the cell after binding to the structure.

In a preferred embodiment of the pharmaceutical composition contains an amount of the oligonucleotide which is effective to inhibit the expression of Id2.

In a seperately preferred embodiment the pharmaceutical composition contains an oligonucleotide which is coupled to a substance which inactivates mRNA. Examples of such "substances" include, but are not limited to, ribozymes. In this embodiment the pharmaceutically acceptable carrier may be capable of binding to a receptor on a cell capable of being taken up by the cell after binding to the structure. In this embodiment the pharmaceutical composition the pharmaceutically acceptable carrier may additionally be capable of binding to a receptor which is specific for a selected tumor cell type.

This invention also provides a method of inducing terminal differentiation in a tumor cell which comprises contacting the tumor cell with the antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA. Tumors cells against which the antisense oligonucleotides would be useful may be chosen from the group consisting of rhabdomyosarcoma, neuroblastoma, and cancerous tumors of the brain and central nervous system.

Finally, this invention provides a method of treating a tumor in a subject which comprises administering the

antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA. In one embodiment, the method comprises administering the pharmaceutical composition defined above to the subject.

In the practice of this invention, the administration of the antisense oligonucleotide or a pharmaceutical composition comprising the antisense oligonucleotide may be effected by any of the well known methods including, but not limited to, oral, intravenous, intraperitoneal, intramuscular or subcutaneous or topical administration. Topical administration can be effected by any method commonly known to those skilled in the art and include, but are not limited to, incorporation of the pharmaceutical composition into creams, ointments or transdermal patches.

In the practice of the above defined method the tumor may be chosen from the group consisting of rhabdomyosarcoma, neuroblastoma, and cancerous tumors of the brain and central nervous system.

The following Experimental Details are provided to aid in the understanding of the invention. The Experimental Details are not intended, and should not be interpreted, to limit the scope of the invention which is more fully defined in the claims which follow.

Experimental Details

Example 1: Id overexpression in RMS tumor cell lines.

The overexpression of Id in rhabdomyosarcoma (RMS) cell lines was examined by the following Northern analysis. Cytoplasmic RNA was isolated from RMS cell lines and normal primary human myoblasts by lysing the cells in 0.5% NP-40 and pelleting the nuclei by low speed centrifugation. The cytoplasmic supernatant was extracted immediately in a 1:1 solution of phenol/chloroform, repeated and the nucleic acids precipitated in 75% ethanol. The yield of cytoplasmic RNA was quantitated by UV absorbance at 260nm and checked by electrophoresis on a 1.5% agarose gel. In order to quantitate the levels of ID mRNA in each of the samples, 5 micrograms of cytoplasmic RNA was electrophoresed on a 1.5% TBE-formaldehyde gel and transferred to a nylon membrane by standard protocols. The nylon filter was then incubated at 58°C in Church/Gilbert buffers (Church and Gilbert, 1984) and Id probes made radioactive by the incorporation of ^{32}P nucleotide precursors in a standard nick-translation protocol (Maniatis et al., 1982). After a 48 hour hybridization period, the filters were washed at 55°C (8x, 5 minutes per wash) in Church/Gilbert buffers without the Id probe, dried and then exposed to film for 16 hours. The bands corresponding to Id mRNA were quantitated by phosphoimage analysis using a Fuji BAS1000 phosphoimager. One RMS cell line, called RH1, demonstrated extremely high levels of Id expression and prompted us to examine Id levels in primary RMS tumor samples.

A Northern analysis of total RNA isolated from 5 different rhabdomyosarcoma (RMS) cell lines under proliferating or differentiation inducing conditions shows clearly that steady-state mRNA levels of Id2 are

significantly up-regulated in one of the lines tested (RH1) relative to normal primary human myoblasts. See Figures 1A through 1C. RH1 cells also showed a dramatic reduction in MyoD and myogenin steady-state mRNA levels (data not shown) consistent with our previous observation that over expression of Id1 in C2C12 cells causes a decrease in mRNA levels of the myogenic regulatory factors. The RH1 cell line was derived from an embryonal RMS as was RH18. The other cell lines tested were derived from alveolar RMS. This limited study, therefore, shows that a subset of embryonal RMS express levels of one Id family member.

Example 2: Analysis of Id expression in RMS primary tumor samples.

For the analysis of Id expression in primary samples, tumor and non-malignant specimens were obtained directly at the operating room by the Tumor Procurement Service at Memorial Sloan-Kettering Cancer Center. These samples had been placed within cold 4% paraformaldehyde-PBS within 20 minutes of excision from the patients. After an overnight incubation at 4°C, the tissues were processed through a series of washings and dehydration steps for embedding in paraffin blocks and then stored at 4°C until sectioning. Eight micron sections have proved to be suitable for this analysis. Prior to hybridization, the tissue sections are deparaffinized and deproteinized to make this tissue more porous to the nucleic acid probes. 100 base pair riboprobes specific for human Id-1,2,3, and sense probes (as a negative control) are being utilized. Probes are incubated with tissue sections for 16 hours at 52°C. After a series of washings, the slides are processed for autoradiography. After dipping in Kodak NTB-2, the slides are incubated for 7 days prior to development. Slides are then scored by 3 investigators. See Table 1.

TABLE 1

	Id1	Id2	Id2-sense	Id3
Patient #				
1	+	+	-	+
2	+	+	-	+
3	+	-	-	+
4	+	+	-	+
Tx Patient #				
1	+	-	-	+
2	+	-	-	+
3	+	-	-	+
4	+	-	-	-
5	+	-	-	+
6	+	-	-	+
7	+	+	-	+
Normal Muscle				
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	-

Patients # 1 through 4 were all diagnosed with RMS and tumor samples taken prior to any chemotherapy or radiation treatment. "Tx patient" refer to tumor samples taken from patients with recurrent RMS after threatment with chemo and/or radiation therapy. "Norm. Muscle" refers to normal muscle tissue. Id1, 2, and 3 columns refer to the analysis of samples stained with antisense probes specific for Id1, 2 and 3, RNA, respectively, by in situ hybridization. An Id2-sense probe was used as a negative control in all hybridizations. All slides were read by three independent viewers and only those that gave a consensus are listed.

Discussion

A majority of RMS tumor samples have been shown to express high levels of the three Id isoforms (9/10 patients examined) whereas the normal muscle samples are negative. Importantly, patients with tumors after chemotherapy and/or radiation no longer express high Id levels which correlates with a more differentiated tumor histology.

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What is claimed is:

1. A method for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor which method comprises:

a) contacting a tumor sample with a nucleic acid probe, wherein the probe is labeled with a detectable moiety and comprises at least 50 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of nucleic acid encoding an Id gene, under conditions permitting the hybridization of the probe to any of the nucleic acid present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any probe not hybridized in the tumor sample; and

c) detecting the presence of any probe hybridized to any of the nucleic acid present in the tumor sample thereby detecting the presence of nucleic acid encoding Id in the tumor sample.

2. The method of claim 1, wherein the Id gene is the Id2 gene.

3. The method of claim 1, wherein the nucleic acid probe comprises between 50 and 300 nucleotides.

4. The method of claim 1, wherein the nucleic acid probe comprises about 150 nucleotides.

5. The method of claim 1, wherein the detectable moiety is a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label or a label which may be detected through a secondary enzymatic or binding step.

6. A method for identifying tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of an Id protein in the tumor which method comprises:

a) contacting a tumor sample with an antibody, wherein the antibody is labeled with a detectable moiety and is capable of specifically binding to an Id protein, under conditions permitting the binding of the antibody to any of the protein present in the tumor sample;

b) treating the tumor sample from (a) under conditions permitting the removal of any antibodies not bound in the tumor sample; and

c) detecting the presence of any antibodies bound to the protein in the tumor sample thereby detecting the presence of an Id protein in the tumor.

7. The method of claim 6, wherein the Id protein is the Id2 protein.

8. The method of claim 6, wherein the detectable moiety is a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label or a label which may be detected through a secondary enzymatic or binding step.

9. A method for <u>in situ</u> identification of tumors which may be susceptible to treatment by chemotherapy or radiation by detecting the presence of nucleic acid encoding Id in the tumor which method comprises contacting the tumor with a suitably labeled nucleic acid reagent capable of detecting an Id gene or gene product.

10. The method of claim 9, wherein the Id gene is the

Id2 gene.

11. The method of claim 9, wherein the suitably labeled nucleic acid reagent is labeled with a detectable moiety chosen from the group consisting of a fluorescent label, a radioactive atom, a paramagnetic ion, biotin, a chemiluminescent label and a label which may be detected through a secondary enzymatic or binding step.

12. An antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA encoding for Id so as to prevent translation of the mRNA.

13. The antisense oligonucleotide of claim 12, wherein the oligonucleotide has a sequence capable of specifically hybridizing to an mRNA encoding for the Id2 gene.

14. A replicable vector comprising the antisense oligonucleotide of claim 12.

15. A host cell containing the vector of claim 14.

16. The host cell of claim 15, wherein the cell is a eukaryotic cell.

17. The host cell of claim 15, wherein the cell is a bacterial cell.

18. The host cell of claim 15, wherein the cell is a mammalian cell.

19. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 12 which is capable of passing through a cell membrane and effective to

inhibit the expression of Id and a suitable pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19, wherein the oligonucleotide is effective to inhibit the expression of Id2.

21. The pharmaceutical composition of claim 19, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

22. The pharmaceutical composition of claim 21, wherein the substance which inactivates mRNA is a ribozyme.

23. The pharmaceutical composition of claim 19, wherein the pharmaceutically acceptable carrier binds to a receptor on a cell capable of being taken up by the cell after binding to the structure.

24. The pharmaceutical composition of claim 23, wherein the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected tumor cell type.

25. A method of inducing terminal differentiation in a tumor cell which comprises contacting the tumor cell with the antisense oligonucleotide of claim 12.

26. The method of claim 25, wherein the tumor cell is chosen from the group consisting of rhabdomyosarcoma, neuroblastoma, and cancerous tumors of the brain and central nervous system.

27. A method of treating a tumor in a subject which comprises administering the antisense oligonucleotide of claim 12 to the subject.

28. A method of treating a tumor in a subject which comprises administering the pharmaceutical composition of claim 19 to the subject.

29. The method of claim 27 or 28, wherein the tumor is chosen from the group consisting of rhabdomyosarcoma, neuroblastoma, and cancerous tumors of the brain and central nervous system.

1/1

FIGURES 1A-1C

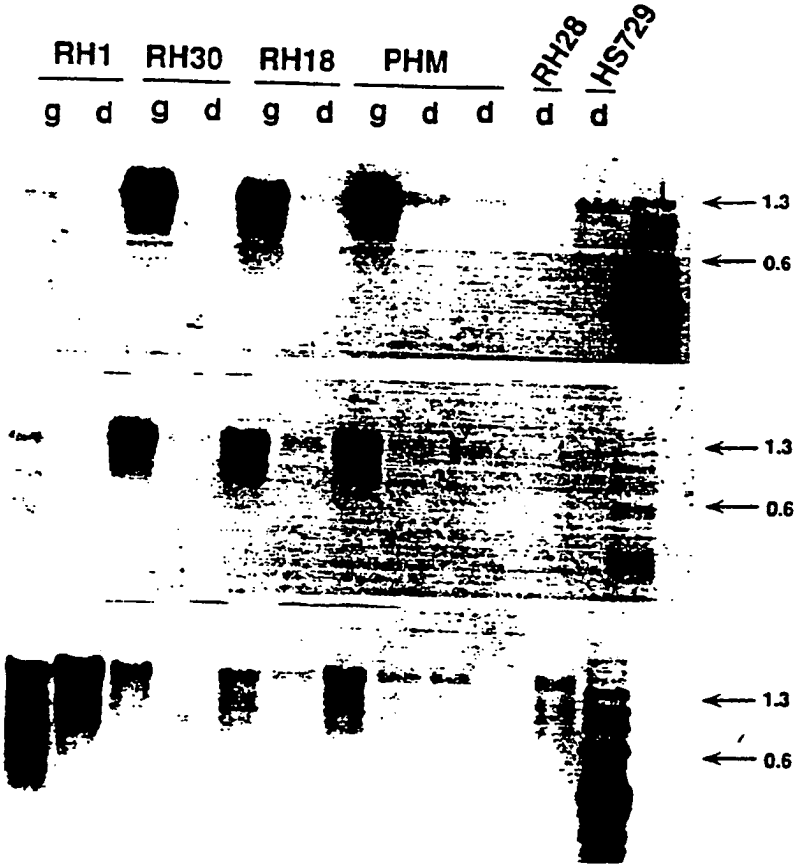

FIGURE 1A

FIGURE 1B

FIGURE 1C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12014

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68; G01N 33/574; C07H 21/04
US CL :435/6, 7.1, 7.23; 514/44; 536/24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 7.23; 514/44; 536/24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, DERWENT, CAS ONLINE
search terms: id, id1, id2, id3, tumor, antisense, cancer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHU et al. Id gene expression during development and molecular cloning of the human Id-1 gene. Mol. Brain Res. June 1995, Volume 30, Number 2, pages 312-326, especially pages 321-323 and 324-325.	1-24
Y	BIGGS et al. A human Id-like helix-loop-helix protein expressed during early development. Proc. Natl. Acad. Sci. USA. February 1992, Volume 89, pages 1512-1517, especially pages 1515-1516.	1-24
Y	MARCUS-SEKURA. Techniques for using antisense oligodeoxyribonucleotides to study gene expression. Analytical Biochemistry. 1988, Volume 172, pages 289-295, see entire document.	12-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 SEPTEMBER 1996

Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12014

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OGATA et al. Expression of ID, a negative regulator of helix-loop-helix DNA binding proteins, is down-regulated at confluence and enhanced by dexamethasone in a mouse osteoblastic cell line, MC3T3E$_1$. Biochem. Biophys. Res. Comm. 14 November 1991, Volume 180, Number 3, pages 1194-1199.	1-29
A	ELLMEIER et al. Mutually exclusive expression of a helix-loop-helix gene and N-*myc* in human neuroblastomas and in normal development. EMBO J. 1992, Volume 11, Number 7, pages 2563-2571.	1-29
A	BENEZRA et al. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. Cell. 06 April 1990, Volume 61, pages 49-59.	1-29